REMARKS

Reconsideration and withdrawal of the rejections set forth in the Office action mailed February 22, 2006 are respectfully requested in view of the amendments and arguments presented herein. A request for a three-month extension of time accompanies this response. The present Amendment is thus timely filed.

I. Amendments

A. Claim Status

Claims 1-67 are canceled.

Claims 68-77 and 95 are withdrawn from further consideration.

Claims 78, 80, 81-85, 87-89, 92-94, and 96 are currently amended.

Claims 97-99 are new.

B. Support for Amendments

Claims 78 has been amended to include the feature of a branched water-soluble polymer structure. Support for this structure is found in numerous locations throughout the specification, and in particular, at page 12, lines 13-32; and on page 13, lines 16-18; page 29, lines 1-5; and in the claims as originally-filed.

Claim 80. Claim 80 recites the feature of identifying certain polymer impurities contained in the composition. The identification of such impurities is supported in the specification on pages 21, lines 1 through page 27, line 22, which describes the preparation and purification of exemplary branched polymers of the invention.

Claims 81-82 have been amended to provide proper antecedent basis consistent with the language of the independent claim.

Claims 83, 84, 87, and 96 have been amended for consistency in language with newly amended claim 78 from which these claims either directly or indirectly depend.

Claim 85 has been amended to recite a particular molecular weight range for the branched polymer, support for which is found in the specification at page 30, lines 1 through 17.

Claim 88 finds support in the specification at page 42, lines 1-2.

Claim 89 finds support in the specification at page 42, lines 1-14.

Claim 92 finds support in the specification, e.g., at page 12, line 13 through page 15, line 12.

Claims 93 and 94 have been amended to change their dependencies.

Claim 96 finds support in the specification at page 12, lines 18-20.

Claim 97 finds support in the specification at page 12, line 27-29.

Claim 99 finds support in the specification at page 21, lines 10-14.

No new matter is introduced into the claims as a result of the amendments presented herein.

II. Kitaguchi et al Document

The Examiner has noted that a copy the Kitaguchi et al document listed as the fifth entry on page 3 of Form PTO/SB/08A mailed on November 10, 2004 was apparently not received in a parent application. Enclosed is a copy of the Kitaguchi reference, along with a copy of Form PTO/SB/08A, as initialed and considered by the Examiner on February 10, 2006. In such document, the Kitaguchi entry is lined through but not initialed by the Examiner. The Examiner is respectfully requested to review the enclosed Kitaguchi publication, and return a copy of Form PTO/SB/08A with the Kitaguchi document entry unlined and initialed by the Examiner.

III. Rejections Under 35 U.S.C. §112, First Paragraph

The Examiner has rejected claims 78-94 and 96 under 35 U.S.C. §112, first paragraph, for failure to comply with the written description requirement. It is submitted that this rejection has been overcome for the following reasons.

Independent claim 78, from which the remaining claims either directly or indirectly depend, has been amended to recite a particular branched polymer structure as recited in the specification and claims as originally-filed: e.g., on page 12, lines 13-32, and in original claim 1 and original claim 18.

In view of the above amendment, it is submitted that claims 78-94 and 96 meet the requirement of 35 U.S.C. §112, first paragraph, since the subject matter recited therein is more than adequately described in the specification as originally-filed. Support for the branched polymer structure is set forth above, while support for the remaining terms and phrases in the claims currently pending was provided in the Preliminary Amendment filed with the application on August 5, 2003.

It is submitted that one skilled in the art, when looking at the subject claims along with the patent specification, would reasonably conclude that the inventors had possession of the invention as embodied in the current claims.

Withdrawal of this rejection is thus respectfully requested.

IV. Rejections Under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected claims 78-94 and 96 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and claim the subject matter which the applicant regards as the invention. In particular, the Examiner has asserted that the claims are confusing and unclear by recitation of the following terms, "a PEG polymer comprises an end capping group and site suitable for interacting with ion exchange chromatographic media, "PEG diol", "end-capped PEG-OH", and "activated end-capped PEG". This rejection is respectfully traversed for the following reasons.

The standard under 35 U.S.C. §112, second paragraph, is that the scope of the claims must be clear to a hypothetical person having an ordinary level of skill in the art. It is submitted that this standard is met by the language of the present claims for the reasons provided below.

Moreover, the Examiner is reminded that, "the words used in the claims are examined from the perspective of a person skilled in the art at the time of the invention, not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification from which it arose, informed as needed, by the prosecution history". *Phillips v. AWH Corp.*, 75 USPQ2d, 1321 (CAFC 2005).

Claim 78, which previously recited the phrase, "*PEG polymer comprising an end-capping group and a site suitable for interacting with ion exchange chromatography media*", has been amended to recite the feature of a particular branched *structure*, such that the metes and the bounds of the claim have been more particularly described. Moreover, the above phrase is now in reference to such structure.

A. <u>Site suitable for Interacting with Ion Exchange Chromatography Media:</u> Regarding the phrase "site suitable for interacting with ion exchange chromatography media", it is submitted that this phrase would be clearly understood by a hypothetical person having an ordinary level of skill in the chemical or polymer arts. As would be understood by such person, ion exchange chromatography is a chromatographic separation technique based on charge. In carrying out such a separation, the stationary phase (media) is either positively or negatively charged, depending upon the molecule to be purified, and the compound to be separated or purified possesses an ionizable group (i.e., a group capable of possessing a charge) capable of interacting with the stationary media employed. Thus, it would be clearly understood by such skilled person that a site suitable for interacting with ion exchange chromatography media is simply an ionizable group.

B. <u>PEG diol</u>. Regarding the term, "PEG diol", the Examiner is directed to page 4 of the specification, lines 10-17. At such location, a definition and structure is provided for the term, "PEG diol".

C. <u>End-capped PEG-OH</u>. Regarding the term, "end-capped PEG-OH", as would be appreciated by one skilled in the chemical or polymer arts, this term refers to a linear PEG molecule having a hydroxyl (i.e., –OH group) at one terminus and an essentially non-reactive or end-capping group at the opposite terminus. The examiner is referred to page 6 of the specification, lines 13-20, in which it is stated, *"it is desirable…to cap the PEG molecule on one end with an essentially non-reactive end moiety… "*. Additional support for the meaning of this term is provided on page 13, lines 16-20 of the

specification, where described is "a PEG moiety capped with an essentially nonreactive end group".

D. "Activated End-Capped PEG". Regarding the preceding term, support for the "end-capped PEG" portion of this term is provided above. As would clearly be appreciated by one skilled in the chemical or polymer arts, "activated end-capped PEG", in particular when such term is viewed in the context of the accompanying specification which clearly discusses the separation of such compounds, an "activated end-capped PEG" is a linear PEG molecule having at one terminus an end-capping group as described above, and at the other terminus, an activated or reactive end-group. The examiner is directed to page 19 of the specification, lines 24-37, which describes polymers said to be "activated".

Moreover, the term "activated" in the context of an activated group or activated polymer is used throughout the specification, e.g., at least at the following locations: at page 10, lines 1-3; page 10, lines 7-12; page 11, lines 31-32; page 14, lines 33-36; page 16, lines 1-2; page 16, line 28 to page 17, line 6; page 17, line 12; page 22, line 16; and so on.

In view of the above, it is submitted that the claims at issue meet the standards of definiteness as set forth under 35 U.S.C. §112, second paragraph. Withdrawal of this rejection is therefore respectfully requested.

V. Prior Art Rejections: Rejections Under 35 U.S.C. §103

A. GROUNDS OF REJECTION.

The Examiner has rejected claims 78-94 and 96 under 35 U.S.C. §103(a) as unpatentable over Martinez et al., U.S. Patent No. 5,643,575, in view of Yoakum (U.S. Patent No. 4,650,909) and El-Tayar et al (U.S. Patent No. 6,638,500), and *if necessary* in further view of Sartore and Veronese, Abuchowski or Seely (U.S. Patent No. 5,935,564).

It is the Examiner's contention that it would have been obvious to use ion exchange chromatography to purify the polymer of Martinez in view of the secondary references to arrive at the method encompassed by the present claims. This rejection, as well as the asserted grounds therefore, are traversed for the reasons set forth below.

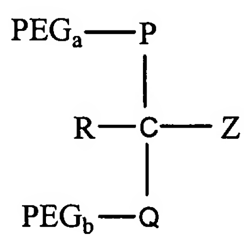
B. <u>THE INVENTION</u>

The present invention is directed to a method for providing a substantially pure branched polymer having the structure shown. Prior to the present invention, branched polymers, and in particular, polymers having molecular weights higher than about 5,000 daltons, were not commonly available in high purity form. (See, for example, the specification at page 7, lines 6-13).

The invention as presently claimed, comprises the following steps:

a. providing an impure polymer composition comprising:

(i) a water-soluble polymer having the structure:



where R is a nonreactive moiety, Z is a moiety comprising a site suitable for interacting with ion exchange chromatography media, PEG_a and PEG_b are each independently an end-capped polyethylene glycol (PEG), and P and Q each comprise a nonreactive linker absent an aromatic ring or ester group, and

(ii) one or more polymeric impurities selected from the group consisting of PEG-diol, end-capped PEG-OH, and activated end-capped PEG, and

(b) purifying said impure polymer composition by ion exchange chromatography under conditions effective to provide the water-soluble polymer in substantially pure form.

B. <u>THE CITED ART</u>.

Martinez (U.S. Patent No. 5,643,575). Martinez is directed to branched, non-antigenic polymers and their conjugates. Although Martinez teaches branched polymer *structures* of the type embodied by the present claims, nowhere does Martinez teach or suggest the presence of one or more polymer impurities of the type claimed by the Applicants, nor does Martinez suggest purifying such a polymer by ion exchange chromatography.

Rather, with respect to activated branched polymers *per se*, Martinez states merely that *"after synthesis, the activated branched polymers can be purified by conventional methods..."* column 7, lines 13-14. In no way does this statement lead one to arrive at the method presently claimed by the Applicant.

An example of one such conventional method of purification (as referred to by Martinez) is recrystallization, as taught in the Martinez examples. Although not recognized by Martinez, the Applicant has shown that recrystallization, while used by Martinez to "purify" the branched polymer reagents described in the '575 patent, is an ineffective method for removing polymer impurities to provide branched PEG polymers in substantially pure form.

Provided herein as evidence are Exhibits A-C. These documents were previously submitted in a related application, U.S. Patent Application No. 10/119,546, in which the Applicant is prosecuting claims directed to particular branched polymers that are essentially pure. However, the contents of such documents are submitted to be relevant to the insufficiency of the Martinez disclosure with respect to the claims currently under examination in the instant application. The Examiner is therefore respectfully requested to make these documents of record in the instant application.

Exhibit A describes a repeat of Example 8 from the Martinez patent.

Exhibit B describes the preparation of PEG-2 Lysine by an alternative route and purification of the crude mixture by recrystallization (as taught by Martinez) and by ion exchange chromatography.

Exhibit C is a copy of a Declaration by Joe Milton Harris summarizing the evidence provided in Exhibits A and B.

Exhibits A-C illustrate the insufficiency of the Martinez document to provide a method for obtaining a branched polymer in substantially pure form.

Further, nowhere does Martinez address or recognize the problems associated with the purification of activated branched polymers, or suggest removal of the problematic polymer impurities claimed by the Applicant by ion exchange chromatography. The so-called "purified" polymers of Martinez are really nowhere near to being pure, and in fact, contain, at least in the Example repeated by the Applicant, approximately 30% combined polymer impurities – a result that was *absolutely unrecognized* in the Martinez disclosure.

Yoakum. Yoakum has absolutely nothing to do with the claims currently under consideration by the Examiner. First, Yoakum is directed to a process for preparing fusion grade PEG, that is to say, PEG for use in genetic transformation and hybridoma applications that is non-cytotoxic. Yoakum has nothing to do with purifying branched PEGs, let alone purifying PEGs such as those encompassed by the present claims. Rather, Yoakum is directed to the purification of *linear* PEG diol (that is, HO-$(CH_2CH_2)_n$-OH) having a molecular weight of from about 1000-6000 to provide sterile PEG for use as a fusion reagent. The purification of Yoakum and the purification issues addressed by Yoakum having to do only with fusion applications, are completely unrelated to the present invention. Moreover, the desired product of Yoakum, linear PEG diol, represents one of the polymeric impurities that the Applicant is *removing* via its claimed method! Thus, the very point of Yoakum, when viewed as a whole, is in absolute contrast to the point of the present invention as embodied by the present claims. Finally, there is motivation provided in either Martinez or Yoakum, when such references are viewed in their entireties, to even combine these two references. Indeed, this reference in no way makes up for the deficiencies of Martinez, nor does such reference in any way support the Examiner's contention that the Applicant's claims are obvious in view thereof.

El Tayar.

Priority. The earliest priority date available to El Tayar is 1998, based on the filing date of the provisional patent application listed on the front page of the '500 patent. In contrast, the instant application claims its earliest priority to U.S. Patent Application No. 08/371,065, filed on January 10, 1995. The next earliest priority date, May 16, 1995, is to Patent Application No. 08/443,383, which is a CIP of the '065 application. The instant specification is identical to that of the '383 application. In view of these priority dates, it is submitted that El Tayar is not applicable as prior art under the provisions of 35 U.S.C §103.

However, if, assuming for the sake of argument, that El Tayar were to be considered as prior art to the instant claims, the disclosure of El Tayar lends no more to the argument for the obviousness of the present claims than do any of the other references cited by the Examiner for the reasons provided below.

El Tayar is directed to site-specific PEGylation of interferon-beta. In particular, El Tayar describes a method for preparing interferon-beta covalently bound to a PEG polymer at a single site, i.e., cysteine-17. El Tayar is not remotely concerned with the problem of providing PEG reagents that are substantially pure, let alone a method for providing substantially pure branched PEG reagents and the problems and impurities associated therewith. While El Tayar desribes purification of a *PEG-polypeptide conjugate* by ion exchange chromatography, among other methods, such a purification is typically aimed at separating non-conjugated protein, and unreacted PEG reagent (and in some cases different protein PEG-mers) from the desired PEG conjugate. Purification of such conjugate mixtures and the aim thereof is *completely different and unrelated* to the problems and particular species relevant to the purification of a branched polymer reagent such as that claimed. El Tayar in no way makes up for the deficiencies of Martinez.

Sartore. The Sartore reference is directed to a synthetic method for introducing an amino acid or peptide spacer between a polymer such as mPEG and a protein. The Sartore reference is absolutely irrelevant to the patentability (i.e., obviousness) of the instant claims. First, the PEGs described by Sartore are *linear*, low molecular weight

mPEGs – mPEG-OH having a molecular weight of either 5000 or 1900 daltons. Sartore describes neither the synthesis nor the purification of a *branched* PEG. Since Sartore has nothing to do with branched PEGs, Sartore can't address the problems associated with polymer impurities which arise during the preparation of branched or multi-armed water soluble polymers such as those embodied by the Applicant's claims. Secondly, the activated PEG of Sartore, containing an amino acid or peptide spacer, mPEG-O-C(O)-(amino acid or peptide)-O-succinimide, was purified merely by precipitation followed by recyrstallization from hot ethyl acetate. Nowhere does Sartore describe the purification of any type of PEG reagent by ion exchange chromatography. Rather, as in the case of El Tayar, Sartore describes merely such a separation carried out *on the resulting conjugate* – a distinction which appears to not be understood or appreciated by the Examiner. That is to say, the synthetic approach of Sartore is completely different from that of the Applicants – since Sartore describes purification and removal of impurities *subsequent* to protein conjugation rather than prior to conjugation. Lastly, the instant claims are not only directed to branched polymers (which Sartore fails to teach or suggest), but also to polymers that comprise a linker *that is absent an ester group*. In contrast, the activated polymers of Sartore clearly comprise an ester linker, e.g., mPEG-**O-C(O)**-(amino acid or peptide)-O-Su, making this reference completely irrelevant to the present claims, since Sartore teaches away from activated polymers such as those claimed.

Veronese. The Veronese reference is directed to modification of enzymes using mPEG. Veronese does not describe the problems associated with purifying branched, activated PEGs. In fact, the illustrative activated PEGs discussed in detail are all *linear* PEGs, not branched PEGs, as required by the instant claims. The problems associated with polymer-derived impurities in activated, branched polymer compositions are more complex than in the corresponding linear polymer compositions.

However, even in reference to use of a linear PEG, mPEG-amino acid (6), Veronese notes the presence of an activated mPEG species that does not react with the spacer. This species then decomposes to mPEG-OH, which is removed in the final step of protein purification. Note that nowhere does Veronese describe, even in reference to a

linear polymer, removal of the activated PEG impurity *prior to coupling* to form a pure activated polymer. Rather, the approach described is the so-called removal of such impurity *after conjugation*, that is, from the PEG-modified protein conjugate. Thus, the approach in Veronese, albeit for a linear activated PEG, actually *teaches away* from the present invention by teaching the removal of polymer impurities from the resulting conjugate, rather than purification of the activated polymer prior to conjugation to provide a purified activated polymer *per se*.

Furthermore, Veronese notes on pages 130-131, in reference to the preparation of mPEG-SOD, not only the presence of a bifunctional non-mPEG impurity, but also small amounts of a higher weight PEG oligomer in the mPEG-5000 starting material, thus confirming the problem of polymer-derived impurities at the time of the Applicant's invention. Note that nowhere does Veronese provide a solution to this problem – but rather describes the need to carefully characterize samples of commercial mPEG to assess for such impurities. The presence of polymer impurities in PEG starting materials was not even *commonly* recognized at the time of the invention by those skilled in the art of conjugation chemistry.

<u>Abuchowski</u>, et al. Abuchowski is directed to the covalent attachment of mPEG to asparaginases using mPEG succinimidyl succinate (mPEG-SS). Nowhere does Abuchowski teach or suggest a method for purifying a polymer reagent, let alone a branched polymer reagent, by ion exchange chromatography. Rather, the polymer reagent of Abuchowski is prepared and purified by repeated precipitation by dropwise addition of petroleum ether to a benzene solution containing the polymer reagent, SS-PEG.

Abuchowski does describe, as in the case of El Tayar, Sartore, and Veronese, separation of unbound SS-PEG from PEG-asparaginase – that is to say, purification of the resulting <u>polymer conjugate</u> by removal/separation of unreacted PEG reagent. Such a separation is completely irrelevant to the claims at issue in the instant application.

Nowhere does Abuchowski point to the use of ion exchange chromatography to purify an activated polymer, let alone a branched polymer, nor does Abuchowski

recognize or even remotely suggest such as approach, let alone removal of polymer impurities such as those recited in the Applicant's claims. Finally, the point of the conjugate separation of Abuchowski is the removal of unbound PEG reagent, i.e., SS-PEG, from the conjugate. In contrast, the point of the method of the present invention is not to remove activated PEG-reagent, but to recover such a reagent in essentially pure form.

Seely.

Priority. The priority date of Seely is March 6, 1996. The Examiner will note that a Certificate of Correction was issued in the '564 patent to correct the priority information in the first paragraph of the specification. An on-line examination of the parent case, now U.S. Patent 5,747,639, confirms its filing date as March 6, 1996. It is the Examiner's contention that Seely is effective prior art since the claimed invention is not disclosed in the parent applications (and by this the undersigned assumes that the Examiner is referring to the earliest-filed application in the series, Application No. 08/371,065, filed on January 10, 1995). However, the second filed case in the present series, U.S. Application Serial No. 08/443,383, was filed on May 17, 1995. The '383 application is a CIP of the '065 application. All other filings in the instant patent family are continuations – thus having an identical specification to the '383 application filed on May 17, 1995, which clearly predates Seely. It is submitted that Seely is not prior art to the present claims. The following remarks and arguments directed to the Seely reference are in no way intended to support the Examiner's contention that the present claims do not find support in earlier-filed patent applications in the instant patent family. However, if one assumes for the sake of argument that Seely is considered to be prior art to the instant claims, then the remarks and arguments apply.

Seely is directed to a process for purifying PEGs. Thus, the problem addressed by Seely is related to the problem addressed by the Applicant's invention. However, the similarity ends there. Seely describes a process for purifying sulfone-activated PEGs using hydrophobic interaction chromatography (HIC), while the claimed invention is directed to a method for purifying branched polymers by ion exchange chromatography (IEC). The polymers of Seely are PEGs having a reactive sulfone group, i.e., a sulfone to

which a two carbon group is bonded (col 5, lines 8-11). (Such PEGs are useful for selective coupling to thiol groups). One such example is PEG vinyl sulfone, PEG-SO_2-$CH=CH_2$. Sulfone groups are not ionizable - thus the molecules of concern to Seely are distinctly different from those molecules recited in the instant claims, i.e., branched polymers comprising a site suitable for interacting with ion exchange chromatography media. Moreover, hydrophobic interaction chromatography is a completely different chromatographic technique from ion exchange chromatography. HIC is a technique that is based on differences in molecules' surface hydrophobicity while IEC separates molecules based on differences in their anionic or cationic charge characteristics. The contention that Seely contributes to the obviousness of the claimed invention is absolutely unfounded. Moreover, to modify Seely to arrive at the Applicant's claimed method would make Seely inoperable for its intended use – since ion exchange chromatography would be ineffective in separating/purifying a molecule such as a polymer-sulfone.

Finally, it should be noted that Seely is instructive in terms of its commentary on the state of the polymer art around the time of the Applicant's invention. Although it is the Examiner's contention that it would have been obvious to modify Martinez to arrive at the Applicant's claims, Seely clearly points to exactly the opposite. Seely states,

"A problem encountered by those skilled in the art when producing compounds such as the c105 TNFBP mutein dumbbell is that the procedures to synthesize the 20 K PEGbv, or other polymer, have several drawbacks, depending upon the synthesis procedure used. One particularly problematic drawback is that the resultant 20K PEGbv will often contain high molecular weight PEG impurities and PEG mono-vinylsulfone. These impurities can then adversely affect the overall yield and purity of the desired dumbbell compound. The need exists, therefore, for methods which would further purify the PEGbv, or other polymers, and thereby enhance the overall purity and yield of the desired ...compound, and make utilization of such compounds as therapeutic agents more commercially practicable." Col 2, lines 30-44. Seely further describes knowing of no preparative chromatographic procedures for successfully separating PEGs. (Col 2, lines 45-56). Seely also describes dramatic differences in overall yield and purity of the

resulting conjugates prepared using HIC-purified PEGbv versus conjugates prepared using non-HIC purified PEGbv. This document is instructive as to the state of the polymer art at the time of the invention, since Seely has a filing date that is **after** that of the Applicant's.

C. ARGUMENT

Based on the remarks above, it can be seen that in no way does the prior art, when considered singly or in combination (regardless of the improper nature of such combination) suggest the steps comprising the Applicant's claimed method. Specifically, nowhere does the prior art suggest purifying a branched polymer of the typed claimed by ion-exchange chromatography to arrive at a substantially pure polymer, nor does the prior art suggest removing the impurities effectively removed by the Applicant's method.

The impurities arising from the synthesis of a branched polymer are not recognized or detected by Martinez – that is to say, the problem addressed by the present invention is not even recognized in the primary Martinez reference. It is completely illogical to argue that it would have been obvious to remove impurities from a polymer reaction mixture to arrive at a pure polymer, if such impurities were undetected/unrecognized in the first place.

The Examiner has argued that it would be within the level of one of ordinary skill in the art to use convention purification methods that provide greater purity, although Martinez provides no motivation to do so. It is submitted that the Examiner has not met his burden showing that there was even recognition in the art of problematic polymer impurities arising during the synthesis of a branched polymer, let alone an obvious way known in the art to separate such impurities prior to conjugation to a peptide or protein, or a reasonable expectation of achieving a pure activated branched polymer of the type claimed. Indeed, all of the secondary references relied upon by the Examiner in support of his assertions are directed to linear polymers, not branched polymers. In summary, it is submitted that the Examiner has failed to provide a basis for which to logically conclude that one having ordinary skill in the art would have found it obvious, in view of Martinez in combination with Yoakum and El Tayar, and optionally, further in view of

Sartore, Veronese, Abuchowski, or Seely, to arrive at the Applicant's claimed method for purifying a branched polymer, in the absence of the teachings of the Applicant.

In view of the above, withdrawal of the rejection of claims 78-94 and 96 under 35 U.S.C. 103(a) is respectfully requested.

VI. Double Patenting

The Examiner has rejected claims 78-94 and 96 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-49 of U.S. Patent No. 5,932,462.

In response to this rejection, enclosed herein is a timely filed executed terminal disclaimer in compliance with 37 CFR §1.321(c) or §1.321(d). In view of the enclosed executed terminal disclaimer, it is submitted that the rejection of claims 78-94 and 96 on the ground of nonstatutory obviousness-type double patenting has been overcome.

VII. Provisional Double Patenting

The Examiner has provisionally rejected claims 78-94 and 96 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 96-109, 111, 112, and 114-133 of co-pending Application No. 10/119,546.

The Examiner is respectfully requested to hold this rejection in abeyance until such time as the conflicting claims have in fact been patented.

VIII. Conclusion

In view of the foregoing, the Applicant submits that the claims pending in the application meet the requirements of 35 U.S.C. §112 first and second paragraphs and patentably define over the art of record. A Notice of Allowance is therefore respectfully requested.

If a telephone conference would expedite the prosecution of the subject application, the Examiner is requested to call the undersigned at (650) 493-3400.

Respectfully submitted,

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Date: August 22, 2006

Form PTO/SB/08A (Modified)	Atty Docket No.: SHE0010.13	Application No.: 10/634,970
Information Disclosure Statement By Applicant	Applicant: J. Milton Harris et al.	
(Use Several Sheets if Necessary)	Filing Date: 08/05/2003	Group: 1713

U.S. Patent Documents

Examiner Initial	No.	Patent No.	Date	Patentee	Class	Sub-Class	Filing Date
	*	4,179,337	12/18/1979	Davis et al.			
	*	4,722,906	02/02/1988	Guire			
	*	5,122,614	06/16/1992	Zalipsky			
	*	5,168,057	12/01/1992	Oh et al.			
	*	5,183,660	02/02/1993	Ikeda et al.			
	*	5,324,844	06/28/1994	Zalipsky			
	*	5,359,030	10/25/1994	Ekwuribe			
	*	5,438,040	08/01/1995	Ekuwribe			
	*	5,605,976	02/25/1997	Martinez et al.			
	*	5,643,575	07/01/1997	Martinez et al.			
	*	5,681,567	10/28/1997	Martinez et al.			
	*	5,756,593	05/26/1998	Martinez et al.			
	*	5,919,455	07/06/1999	Greenwald et al.			
	*	6,113,906	09/05/2000	Greenwald et al.			
	*	6,362,254	03/26/2002	Harris et al.			
	*	6,437,025	08/20/2002	Harris et al.			
	*	6,664,331	12/16/2003	Harris et al.			
	*	2002/0052430	05/02/2002	Harris et al.			
	*	2002/0052443	05/02/2002	Greenwald et al.			
	A	2003/0114647	06/19/2003	Harris et al.			

Examiner:	Date Considered: 2/11/06

Examiner: Initial citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO/SB/08A (Modified)	Atty Docket No.: SHE0010.13	Application No.: 10/634,970
Information Disclosure Statement By Applicant	Applicant: J. Milton Harris et al.	
(Use Several Sheets if Necessary)	Filing Date: 08/05/2003	Group: 1713

COPY

Foreign Patent or Published Foreign Patent Application

Examiner Initial		No.	Document No.	Publication Date	Country or Patent Office	Class	Sub-class	Translation Yes	No
GM		*	0 400 472	12/05/1990	EP				
GM		*	0 400 486	12/05/1990	EP				
GM		*	0 473 084	03/04/1992	EP				
GM		*	0 632 082	01/04/1995	EP				
GM		*	94/26778	11/24/1994	WO				
GM		*	95/11924	05/04/1995	WO				

Examiner:	Date Considered: 2/10/06

Examiner: Initial citation considered. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

Form PTO/SB/08B (Modified)	Atty Docket No.: SHE0010.13	Application No.: 10/634,970
Information Disclosure Statement By Applicant	Applicant: J. Milton Harris et al.	
(Use Several Sheets if Necessary)	Filing Date: 08/05/2003	Group: 1713

COPY

Other Documents

Examiner Initial		No.	Author, Title, Date, Place (e.g. Journal) of Publication
GM		*	ABUCHOWSKI ET AL., "Cancer Therapy with Chemically Modified Enzymes. I. Antitumor Properties of Polyethylene Glycol-Asparaginase Conjugates," Cancer Biochem. Biophys. (1984), 7:175-186.
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Other Documents

Examiner Initial		No.	Author, Title, Date, Place (e.g. Journal) of Publication
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ENZYMATIC FORMATION OF AN ISOPEPTIDE BOND INVOLVING THE ε-AMINO GROUP OF LYSINE

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Summary: Bacterial protease and lipase act as regioselective preparative catalysts of peptide bond synthesis in anhydrous organic solvents: when a derivative of lysine is used as the amino component, only its ε-NH_2 group reacts (to give an isopeptide linkage).

There is a substantial interest in peptides containing unnatural linkages, i.e., those involving functional groups located in the amino acid side chains, for such structurally unusual peptides may have a number of attractive properties.[1] In particular, the biological characteristics of ε-substituted lysine derivatives are quite distinct from those of the "normal" α-isomers.[2] Enzymatic peptide synthesis has a number of virtues in terms of mild reaction conditions, minimal protection and activation requirements, absence of racemization, and selectivity.[3] It has been invariably assumed that enzymes link the α-carboxyl group of one amino acid to the α-amino group of another; the possibility of the involvement of side chains is usually not even discussed.[4,5]

Kullmann[5] reported the use of α-chymotrypsin as a catalyst in the reaction between N-Boc-L-Tyr-OEt and L-Lys-N_2H_2Ph and implied that only the natural peptide bond (i.e., involving the α-NH_2 group of lysine) was formed. We investigated a similar reaction, between N-Ac-L-Phe-OEt and L-Lys-O-tert-Bu, under analogous conditions (4.7 mL of 0.14 M aqueous carbonate buffer (pH 10.0) containing 32% dimethylformamide, 0.5 and 1.0 mmol of the substrates, respectively, and 4 mg of chymotrypsin, 20°C) and found that in fact after a 1-hr incubation, when the former substrate was exhausted, the ratio of α to ε dipeptide was only 7:3, as determined by HPLC analysis (Waters μBondapak C_{18} column; 30:70 acetonitrile-aqueous buffer (phosphate-triethylamine, pH 3.6) as eluent, 1 mL/min; detection at 220 nm).

In contrast, another protease, that from *Bacillus subtilis* (subtilisin Carlsberg), afforded an overwhelming (>99%) selectivity toward the ε-NH_2 group in the aforementioned peptide synthesis under the same conditions. Unfortunately, the degree of conversion in this reaction never exceeded 30% because of the competing enzymatic hydrolysis. This problem, however, was eliminated when anhydrous tert-amyl alcohol was employed as the reaction medium for subtilisin-catalyzed peptide synthesis[6]: 2.5 mmol of N-Ac-L-Phe-OEtCl and 3.0 mmol of L-Lys-O-tert-Bu were dissolved in 50 mL of the solvent, followed by the addition of 170 mg of the enzyme (lyophilized from pH 7.8[7]), and the suspension was shaken at 45°C for 24 h. The product,[8] obtained with an 85% isolated yield, was pure by HPLC and ^{1}H NMR, and was found to be[9] exclusively the ε-isomer of N-Ac-L-Phe-L-Lys-O-tert-Bu.

Similar data were obtained when a non-protease, Pseudomonas sp. lipoprotein lipase, w used as a catalyst of peptide synthesis[10] in anhydrous toluene: 0.57 g of the pure (by HPLC and ^{1}H NMR) ε-isomer of the dipeptide was prepared with a 51% isolated yield.[11] It is wort noting that when the Phe and Lys amino acid derivatives (0.1 M each) were non-enzymatically reacted in tert-amyl alcohol at 70°C for 4 days, only a 28% conversion was achieved with th ratio of ε to α isomer of 5:1.

Therefore, subtilisin and lipase (both commercially available enzymes), when used in anhydrous organic solvents, are regioselective practical catalysts of peptide synthesis and with lysine as a nucleophile afford the unnatural, ε peptide linkage. Interestingly, this peptide bond is much more stable toward proteolysis than its α-isomer: after a 1-hr incubati with chymotrypsin (0.2 mg/mL) in aqueous solution (pH 7.6, 10% CH_3CN) at 23°C, 76% of the α-isomer of the Phe dipeptide (10 mM) described above was hydrolyzed, while the ε-dipeptide was completely resistant to the enzymatic hydrolysis under these conditions.

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8. Obtained as a crystalline solid with mp 86-88°C, $[\alpha]_D^{25}$ +19.8° (c 0.4, MeOH). Anal. Calcd for $C_{21}H_{33}N_3O_4$: C, 64.42; H, 8.50; N, 10.73. Found: C, 64.66; H, 8.35; N, 10.67.
9. Structure determination was accomplished by 250 MHz ^{1}H NMR. As inferred from the NMR spectrum of independently synthesized N-Ac-L-Phe-α-L-Lys-O-tert-Bu (using chymotrypsin as a catalyst in water[5] with N-ε-CBZ-L-Lys-O-tert-Bu as the nucleophile), formation of the peptide bond through the α-NH_2 group resulted in a downfield shift of the α-proton from 3.31 to 4.27 ppm (and no appreciable effect on the ε-protons). In contrast, the peptide synthesized using subtilisin in tert-amyl alcohol showed a downfield shift only for the ε-protons from 2.74 to 3.08 ppm (and no other significant changes in the NMR spectrum of the dipeptide's lysine moiety).
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11. The same conditions as for subtilisin, except that the alanine rather than phenylalanine ester was used and the reaction catalyzed by 100 mg/mL lipase was carried out for 3 days. The oily product, purified by silica gel column chromatography, was converted to a HCl salt and crystallized (mp 172-173°C (dec), $[\alpha]_D^{25}$ -6.2° (c 0.4, MeOH)). Anal. Calcd for $C_{15}H_{30}ClN_3O_4$: C, 51.20; H, 8.59; N, 11.94. Found: C, 51.17; H, 8.64; N, 11.83.

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